BCA PROTEIN ASSAY IN CONJUCTION WITH THE COMPAT-ABLE PROTEIN ASSAY PREPARATION PROTOCOL

This protocol is adapted from Pierce's BCA Protein Assay Kit by the Gene Expression Lab.
This protocol is for use with Pierce's BCA Protein Assay Kit & Compat-Able Protein Assay Preparation Reagent Set. For additional technical inquiries, contact Technical Service at 800-874-3723 or www.piercenet.com

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BEFORE STARTING THE EXPERIMENT
COMPAT-ABLE PROTEIN ASSAY PREPARATION PROTOCOL
BCA PROTEIN ASSAY

BEFORE STARTING THE EXPERIMENT

- Use the following formula to determine the total volume of WR required: (# standards + # unknowns) x (# replicates) x (volume of WR per sample) = total volume WR required
  **Example: for the Standard Test Tube Protocol with 3 unknowns and 2 replicates of each sample: (9 standards + 3 unknowns) x (2 replicates) x (2 mL) = 48 mL WR required
    a. Prepare WR by mixing 50 parts of BCA™ Reagent A with 1 part of BCA™ Reagent B (50:1, Reagent A:B). For the above example, combine 50 mL of Reagent A with 1 mL of Reagent B.
- Dilute samples accordingly, so that the amount of protein measured per sample is within the boundaries of the standard curve.

Compat-Able Protein Assay Preparation Protocol

NOTE: Be sure to pre-treat the protein standards to be used later in the protein assay exactly the same as the samples to be analyzed

1. In duplicate, dispense 100 µL of each sample or diluted protein standards to be treated into a test tube
2. Add 500 µL of Compat-Able Protein Assay Preparation Reagent 1 to each tube. Mix each tube and allow the tubes to stand at RT for at least five minutes.
3. Add 500 µL of Compat-Able Protein Assay Preparation Reagent 2 to each tube. Mix each tube and centrifuge at 10,000G for at least 5 minutes
4. Invert the tube and discard the supernatant. Blot the open end of the inverted tube on clean paper toweling to completely remove the

supernatant. If needed, a pipette can be used to carefully remove excess liquid.
    b. NOTE: The protein pellet may be difficult to see as it may form a thin layer on the walls of the tube.
5. Dissolve the protein pellet in the original sample volume (100 µL) of ultrapure water or the BCA Working Reagent. Vortex vigorously to solubilize the pellet.

BCA Protein Assay

1. Add 2.0 mL of the WR to each tube and mix well. Cover and incubate tubes at 37°C for 30 minutes
    a. Use a water bath to heat tubes for either Standard (37°C incubation) or Enhanced (60°C incubation) Protocol. Using a forced-air incubator can introduce significant error in color development because of uneven heat transfer.
2. Cool all tubes to RT and then mix end over end.
3. With the spectrophotometer set to 562 nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples within 10 minutes.

Note: Because the BCA™ Assay does not reach a true end point, color development will continue even after cooling to RT. However, because the rate of color development is low at RT, no significant error will be introduced if the 562 nm absorbance measurements of all tubes are made within 10 minutes of each other.

4. Subtract the average 562 nm absorbance measurement of the Blank standard replicates from the 562 nm absorbance measurement of all other individual standard and unknown sample replicates.
5. Prepare a standard curve by plotting the average Blank-corrected 562 nm measurement for each BSA standard vs. its concentration in µg/ mL. Use the standard curve to determine the protein concentration of each unknown sample.
